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METABOLIC RATES IN FIVE ANIMAL POPULATIONS IN 1976 AFTER PROLONGED EXPOSURE TO SEAFARER ELF ELECTROMAGNETIC FIELDS IN NATURE

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FOREWORD

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The author thanks Noreen Ash, Vytas Bindokas, and Dr. Marc Klowden for technical assistance, and J. R. Gauger and W. F. Lancaster of the field staff of IIT Research Institute (IITRI) for electric and magnetic field measurements.

Respectfully submitted,

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1. SUMMARY

Five species of woodland animals were collected during summer, 1976, under the U.S. Navy's Extremely Low Frequency (ELF) antennas at the Wisconsin Test Facility (WTF) and were tested for oxygen consumption and respiratory quotient (R. Q.). Controls were collected 7 to 13 miles from the WTF antenna on the same or the next day and were tested at the same time as the test group. The species tested comprise four invertebrates and one vertebrate. They are: the earthworm, Lumbricus terrestris L.; the redworm, Lumbricus rubellus Hoffmeister; the woodlouse, Oniscus asellus L.; the slug, Arion sp.; and the redbacked salamander, Plethcdon cinereus cinereus (Green). There were no significant differences in the oxygen consumption and R. Q. in any species except the redworm. There was a highly significant ($0.005 > P > 0.001$) elevation in the oxygen consumption of exposed redworms. Our field observations and sampling of the five exposed populations continue to show no abnormalities in behavior, habitat selection, or external features and pigmentation after seven years of WTF operation.

2. INTRODUCTION

In summer, 1972, a program was initiated to monitor the metabolic rates (via oxygen consumption) and the respiratory quotients (R. Q.) of animals living under or near the extremely low frequency (ELF) Seafarer antennas at the Wisconsin Test Facility (WTF). The five species selected for study share the desireable attributes of a sedentary life on or in the soil which favor maximum exposure to the horizontal electric fields and magnetic flux of the antennas. The animals are: the earthworm, Lumbricus terrestris L.; the redworm, Lumbricus rubellus (Hoffmeister); the woodlouse, Oniscus asellus L; the slug, Arion sp.; and the redbacked salamander, Plethodon cinereus cinereus (Green).

The monitoring program has continued each year with the objective of disclosing significant and sustained alterations in metabolism or respiratory quotient after prolonged exposure. Thus far, there have been none (Greenberg, 1974; Greenberg and Ash, 1976a, b). The results of the 1976 monitoring program, after seven years of ELF antenna operation, are presented here.

3. MATERIALS AND METHODS

3.1 Collection of Animals

Exposed animals were collected at the same site used in previous years (Greenberg and Ash, 1976a, b), along the north leg of the antenna, just south of Highway 77. Collection dates of exposed animals are: earthworms, July 18; redworms, August 24; woodlice, June 21; slugs, August 16; and salamanders, July 20. Controls were collected within a day of the exposed animals from the same sites as in previous years (Fig. 1). These sites are 7 to 13 miles from the nearest WTF antenna. Both groups were separately packed in wet moss, placed in an ice chest, and transported by air (2 h, unpressurized Beech 99 jetprop flying at 8-9,000 ft) or by car (woodlice, salamanders, redworms - 8 h) to our laboratory in Chicago where they were weighed and tested immediately or stored at 12°C and tested within 24 hours. Exposed and control animals of the same species were always tested simultaneously.

3.2 Oxygen Consumption and Respiratory Quotient

Oxygen consumption was measured in a closed manometric system with a Gilson differential respirometer set at 20°C. Animals were washed, dried, and weighed to the nearest 0.01 gm and were then placed in 15-ml Warburg flasks with two sidearms. Salamanders were tested one per flask; others were grouped as follows to increase reliability of the system: woodlice, 10; redworms, 3; earthworms, 3; and slugs, 3. Specimens were

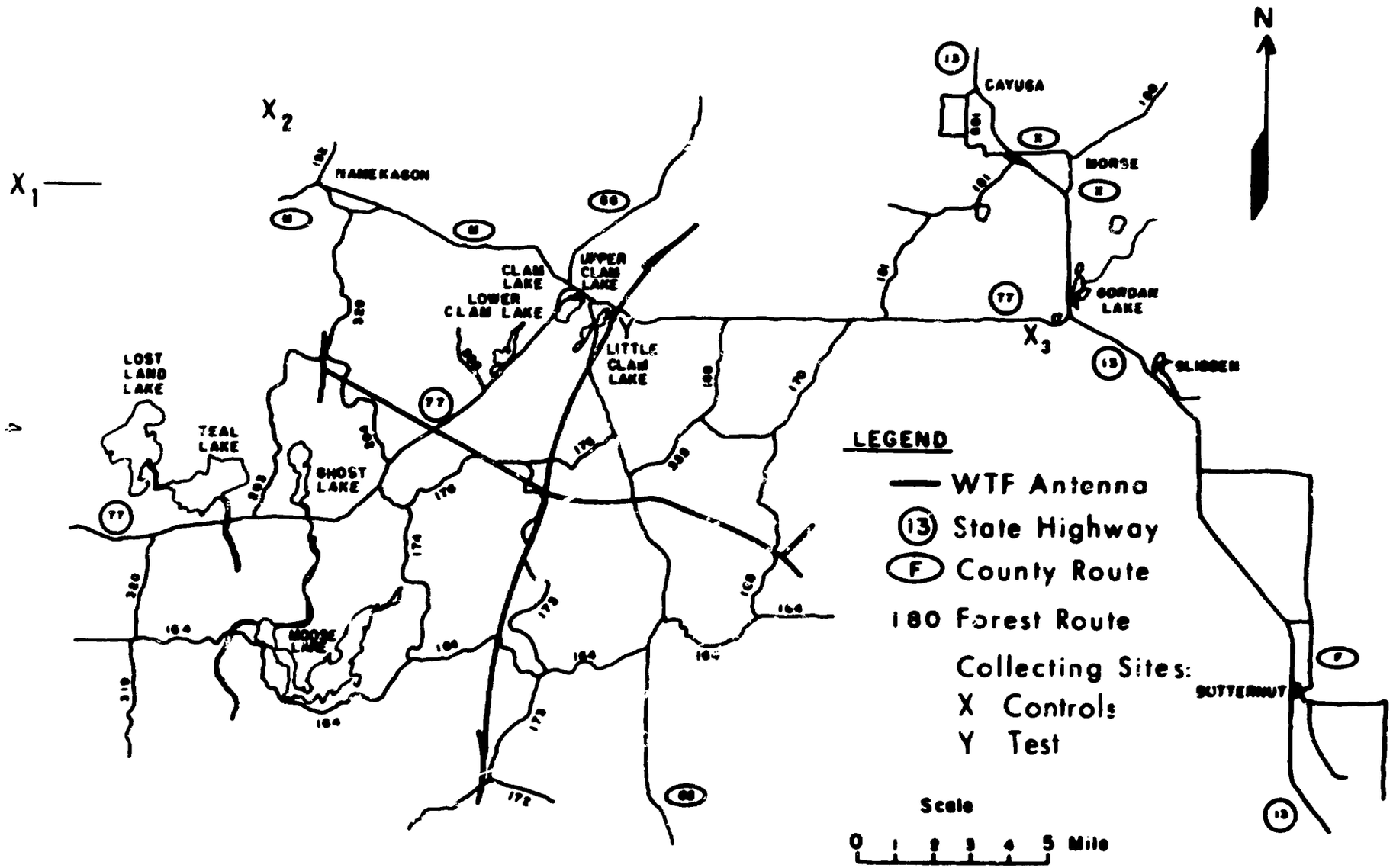

Fig. 1. Collection Sites of Exposed and Control Animals

grouped for uniformity of weight. Woodlice and salamanders were not sexed; earthworms and slugs are hermaphroditic. One-tenth ml of a .5M KOH solution was introduced into the side-arm as a CO_2 absorbent. The center well was partly filled with water to raise the humidity in the flask and screened to exclude the animals. The flasks were allowed to equilibrate at 20°C for 15 minutes. When volumes of O_2 consumption were roughly equal for two consecutive periods, readings were taken for an additional two or three periods, totalling four or five consecutive periods as a basis for mean oxygen consumption. Respiration rate is expressed as µl O_2 consumed/gm/h. Volume of carbon dioxide evolved during respiration was determined by titration of 0.2N HCl against the alkaline absorbent plus 1 ml saturated Ba Cl_2 and 1 ml 0.1% methyl orange. µl CO_2 was calculated using the gas laws to obtain respiratory quotient (RQ).

3.3 Electric and Magnetic Field Measurements

The equipment employed for the electromagnetic field measurements described here were the specially constructed tuned voltmeters (TVM's) which were supplied by the Navy for ELF measurements, the commercially available Hewlett-Packard 302A wave analyzer, and the commercially available Hewlett-Packard 3581A signal wave analyzer. All three meters are battery operated. The TVM's and HP302A were used from 1972 through 1975, and the HP3581A was first used in 1976. The HP3581A is a newly available

instrument, and was factory modified for a 1 Hz bandwidth and battery operation. It is being used to replace the increasingly unreliable TVM's and the bulky HP302A.

The magnetic field was measured using a single axis magnetic field probe designed and built by IITRI. This probe is merely a many-turn coil with a ferrite core and terminating resistor. In each case, the field was measured in three perpendicular directions (north-south, east-west, and vertical), and the root of the sum of the squares was taken for each antenna condition. Appropriate conversion factors were used to convert the voltage reading at the output of the probe to an equivalent magnetic flux density. The 60 Hz values were measured with the antenna off.

The low impedance electric field (i.e., the horizontal electric field at the earth's surface) was measured with one-meter probe wires. Two perpendicular components of the horizontal electric field were read and the square root of the sum of the squares was calculated.

3.4 Wisconsin Test Facility Operations

Since March, 1971, the Wisconsin Test Facility has been operated with 300 amperes in either the north-south or east-west antenna, or in both antennas simultaneously. In previous years, the operating schedule was roughly 5 days/week, 6 hours/day, at 42 or 45, 75 or 76 Hz. The schedule for June 1975 to June 1976 is summarized in Table 1 including monthly hours of operation, frequencies employed, and hours of modulated trans-

mission. On August 20, 1976, 24-hour, around-the-clock operation of both antennas was initiated.

3.5 Statistical Treatment

The O_2 consumption data and R.Q. data were analyzed using a single classification analysis of variance for unequal sample sizes. The O_2 consumption data were tested across the years utilizing a mixed model, two-level nested analysis of variance for unequal sample sizes. The 95 percent confidence limits for the mean were computed using t and the standard deviation of the mean.

Table 1. WTF Operation for June 1975 Through May 1976.

Hours of Operation/Month

	Antenna						
	42 or 45 Hertz			75 or 76 Hertz			
Month/Year	E/W	N/S	Both	E/W	N/S	Both	NSB
June 1975	19.5	19.5	1.5	21.5	21.5	16.	---
July	6.35	6.35	--	13.5	13.5	132.	---
August	--	--	--	21.0	--	147.	---
September	--	--	--	5 MOD	--	86.5 + 5 MOD	---
October	--	--	31.5	36 + 85 MOD	36 + 85 MOD	69.	10.5*
November	--	--	--	--	--	236.5	---
December	--	--	--	38.5	96.5	--	---
January 1976	--	--	--	--	--	--	--
February	--	--	--	--	--	--	--
March	--	--	--	31 MOD	--	184.	--
April	--	--	--	32.5 MOD	--	144.	--
May	--	--	--	62 MOD	--	--	--

E/W = East/West

N/S = North/South

Both = East/West and North/South

NSB = North/South Buried

MOD = Modulated Output

Antenna(s) at 300 A unless otherwise noted.

*Antenna at 100 A.

4. RESULTS

4.1 Electric and Magnetic Field Data

Table 2 gives the measured magnetic flux densities at the sites where exposed and control animals were collected for metabolic measurements during the period 1972-1976. The magnetic fields at the test sites reach a maximum of 0.25 gauss (when the north-south antenna is energized) while all control sites are <0.001 gauss. The values of the measured magnetic field show a certain degree of fluctuation from year to year. The reason for this is that the only location where the field exceeds the 0.001 gauss level are physically near an antenna. Near the antenna, the magnetic fields may be expected to vary as the inverse of the distance from the test point to the antenna. As a result of this behavior, the highest values of magnetic field occur closest to the antenna. At these locations, the positioning of the probe is most critical. This fact is borne out by measurement. The higher magnetic flux densities show a larger degree of variation than the lower magnetic flux densities. This reflects the difficulty in obtaining exactly the same measurement locations and positioning of the probe year after year.

Table 3 gives the measured low impedance electric fields at the collecting sites for the period 1972-1976. The 45- and 75-Hz readings show some fluctuation that may be explained, in part, by uncertainty in placement of the sensor. However,

since the electric field varies as the natural logarithm of the inverse of the distance from the antenna, this cannot account for all of the differences from year to year. The main explanation for the yearly variations in these data is that the low impedance electric field is affected by the differences in the earth's conductivity and other factors such as changes in nearby long conductors which occur between measurements. These factors probably account for the two- to three-fold differences in the horizontal field measurements at the test site. These differences, however, are trivial compared with the 10-fold to 10,000-fold differences between the test and the several control sites which occur when the north-south antenna is operating at either 45 Hz or 75 Hz. These differences have been maintained at least since March 1971, when the antenna's operating current was raised to its present level of 300 amperes.

TABLE 2

MAGNETIC FIELDS AT TEST AND CONTROL SITES

Antenna Current 300 Amperes

SITE	Magnetic Flux Density (Gauss)									
	E/W Antenna									
	45 Hz					75 Hz				
	1972	1973	1974	1975	1976	1972	1973	1974	1975	1976
Redworm Test Salamander	a	a	a	b	b	a	a	a	b	b
Salamander Control	b	a	b	b	b	b	a	b	b	b
South Roadside Test	b	b	b	b	b	b	b	b	b	b
Earthworm Isopod Test	a	a	a	b	b	a	a	a	b	b
N Yard Control	b	a	b	b	b	b	a	b	b	b
Dam Control	b	a	a	b	b	b	a	a	b	b
M Yard Control	b	a	a	b	b	b	a	b	b	b
Dam Dump	a	a	a	a	b	a	a	a	a	b

Key: a = no measurement taken; b < 0.001 Gauss.

TABLE 2 - Continued

SITE	Magnetic Flux Density (Gauss)														
	N/S Antenna														
	45 Hz					75 Hz					60 Hz				
	1972	1973	1974	1975	1976	1972	1973	1974	1975	1976	1972	1973	1974	1975	1976
Redworm Test Salamander	a	a	a	0.11	0.12	a	a	a	0.10	0.092	a	a	a	b	b
Salamander Control	b	a	b	b	b	b	a	b	b	b	b	a	b	b	b
South Roadside Test	0.15	0.25	0.15	0.14	0.17	0.20	0.25	0.21	0.21	0.189	b	a	b	b	b
Earthworm Isopod Test	a	a	a	0.053	0.062	a	a	a	0.051	0.044	a	a	a	b	b
N Yard Control	b	a	b	b	b	b	a	b	b	b	b	a	b	b	b
Dam Control	b	a	a	b	b	b	a	a	b	b	b	a	a	b	b
M Yard Control	b	a	b	b	b	b	a	b	b	b	b	a	b	b	b
Dam Dump	a	a	a	a	b	a	a	a	a	b	a	a	a	a	b

Key: a = no measurement taken; b < 0.001 Gauss.

TABLE 3

LOW IMPEDANCE ELECTRIC FIELDS AT TEST AND CONTROL SITES

Antenna Current 300 Amperes

SITE	Low Impedance Electric Field Intensity (Volts/Meter)									
	E/W Antenna									
	45 Hz					75 Hz				
	1972	1973	1974	1975	1976	1972	1973	1974	1975	1976
Redworm/Salamander Test	a	a	a	0.0048	0.0059	a	a	a	0.0039	0.0038
Salamander Control	0.0014	a	0.0007	0.0013	0.0013	0.0006	a	b	0.0009	0.0008
South Roadside Test	0.0090	0.0087	0.0082	0.0080	0.0086	0.0099	0.0082	0.0119	0.0103	0.0071
Earthworm/ Isopod Test	a	a	a	0.0050	0.0046	a	a	a	0.0053	0.0039
N Yard Control	0.0012	a	0.0001	0.0002	0.0002	0.0009	a	0.0003	0.0002	0.0002
Dam Control	0.0003	a	a	0.0004	0.0005	0.0002	a	a	0.0003	0.0003
N Yard Control	b	a	b	b	b	b	a	b	b	b
Dam Dump Control	a	a	a	a	0.0002	a	a	a	a	0.0002

Key: a = no measurement taken; b < 0.00005 volts/meter.

TABLE 3 - Continued

	Low Impedance Electric Field Intensity (Volts/Meter)														
	N/S Antenna														
	45 Hz					75 Hz					60 Hz				
	1972	1973	1974	1975	1976	1972	1973	1974	1975	1976	1972	1973	1974	1975	1976
Redworm/ Salamander Test	a	a	a	0.0776	0.0812	a	a	a	0.170	0.101	a	a	a	0.0001	0.001
Salamander Control	0.0013	a	0.0006	0.0012	0.0012	0.0005	a	b	0.0007	0.0007	0.0012	a	0.0010	0.0011	0.0009
South Roadside Test	0.144	0.106	0.130	0.107	0.0930	0.291	0.176	0.306	0.246	0.117	0.0005	a	b	0.0008	0.0001
Earthworm/ Isopod Test	a	a	a	0.0626	0.0915	a	a	a	0.121	0.106	a	a	a	b	b
N Yard Control	0.0009	a	0.0001	0.0002	0.0003	0.0008	a	0.0002	0.0001	0.0003	0.0005	a	0.0042	0.0036	0.0045
Dam Control	0.0004	a	a	0.0004	0.0003	0.0010	a	a	0.0004	0.0002	0.0018	a	a.	0.0017	0.0014
M Yard Control	b	a	b	0.0001	b	b	a	b	b	b	0.0063	a	0.0025	0.0181	0.0176
Dam Dump Control	a	a	a	a	0.0002	a	a	a	a	0.0004	a	a	a	a	0.0008

Key: a = no measurement taken; b = 0.00005 volts/meter.

4.2 Discussion of Biological Data

Table 4 summarizes mean oxygen consumption in µl/g, with 95 percent confidence limits, of exposed and control animals in summer, 1976. There were no statistically significant differences between exposed and control animals except for a highly significant increase in oxygen consumption in exposed redworms. Table 5 summarizes data on oxygen consumption in all animals since 1972. Since then, a total of 48 paired tests have been performed, of which 45 have not shown statistically significant differences in oxygen consumption. Of the two previous ones that did show differences, one was a marginal ($P < 0.05$) increase in oxygen consumption in exposed woodlice in summer 1972 and the other was a marginal decrease in redworms in fall, 1974. As shown in Table 5, the differences between exposed and control populations since 1972 are highly insignificant whereas the same population in the same habitat - exposed or control - shows significant variability over the same period of time (Table 5). In effect, natural variability is greater than any ELF effect. Figures 2-6 depict in graphic form this within-year and between-year range for each group of animals under surveillance.

In interpreting these interyear non-Seafarer fluctuations in metabolism one needs to consider such meterological factors as rainfall and temperature. U. S. Weather Service data on precipitation have been assembled in Table 6, and air temperature data are given in Table 7. Precipitation data are collected by the Service only after snow is off the ground in May. On May 2, 1976 .55" of snow was collected at WTF which we tabulated as .25" of rain. Mean maximum and minimum temperatures are given for the month

TABLE 4

MEAN OXYGEN CONSUMPTION (µl/g) WITH 95% CONFIDENCE LIMITS OF EXPOSED AND CONTROL ANIMALS IN SUMMER 1976[a]

Organism	No.	Exposed animals' mean O_2 consumption	No.	Control animals' mean O_2 consumption
Earthworms[b]	30	113.78 (98.08 - 129.48)	24	124.68 (111.52 - 137.84)
Redworms	39	166.81 (145.86 - 187.76)	54	127.88 (113.19 - 142.57)
Slugs	24	197.73 (177.60 - 217.86)	45	204.28 (183.91 - 224.65)
Woodlice	210	245.33 (234.35 - 256.31)	80	263.73 (225.41 - 302.06)
Salamanders[c]	16	108.53 (95.69 - 121.37)	12	97.04 (83.97 - 110.11)

[a]There were no significant differences between exposed and control animals except for Redworms (.005 > P > .001).

[b]Weight range = 0.4 to 0.83 g per specimen.

[c]Weight range = 0.6 to 1.4 g per specimen.

TABLE 5

MEAN OXYGEN CONSUMPTION (µl/g) OF EXPOSED AND CONTROL ANIMALS IN THE SUMMERS OF 1972, 1973, 1975, AND 1976 (Analysis of Variance)[a]

Organism		1972	1973	1975	1976	Test vs. Control	Years
Woodlice	Test	235	229.80	172.88	245.33	.75 > P > .50	P < .001
	Control	205.86	222.00	170.44	263.73		
Salamanders[b]	Test	97.99	76.25	89.87	108.53	.75 > P > .50	P < .001
	Control	100.98	85.05	76.39	97.04		
Slugs	Test	134.83	117.40	128.04	197.73	P > .75	P < .001
	Control	120.67	118.74	138.88	204.28		
Earthworms[c]	Test	119.67	85.90	123.22	113.78	P ~ .75	P < .001
	Control	123.33	81.49	126.44	124.68		
Redworms	Test	171.62	145.78	145.78	166.81	P > .75	P < .001
	Control	173.59	135.94	163.98	127.88		

[a] In 1974, O_2 consumption was studied in fall but not summer specimens and, therefore, is a different population which cannot be included in this analysis.

[b] Weight range = 0.6 to 1.4 g per specimen.

[c] Weight range = 0.4 to 0.83 g per specimen.

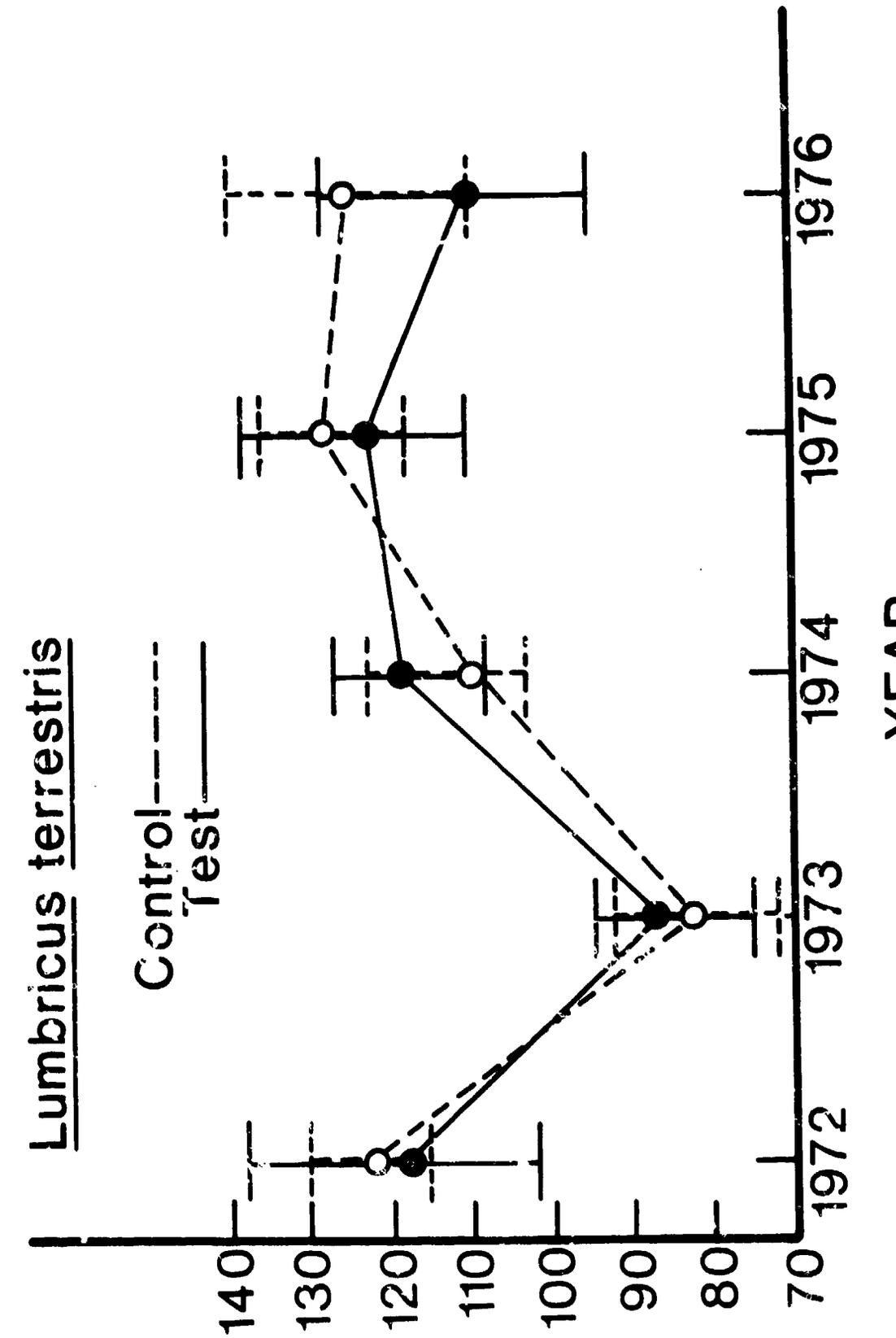

Fig. 2. Mean Oxygen Consumption of the Earthworm, Lumbricus terrestris, 1972-76.

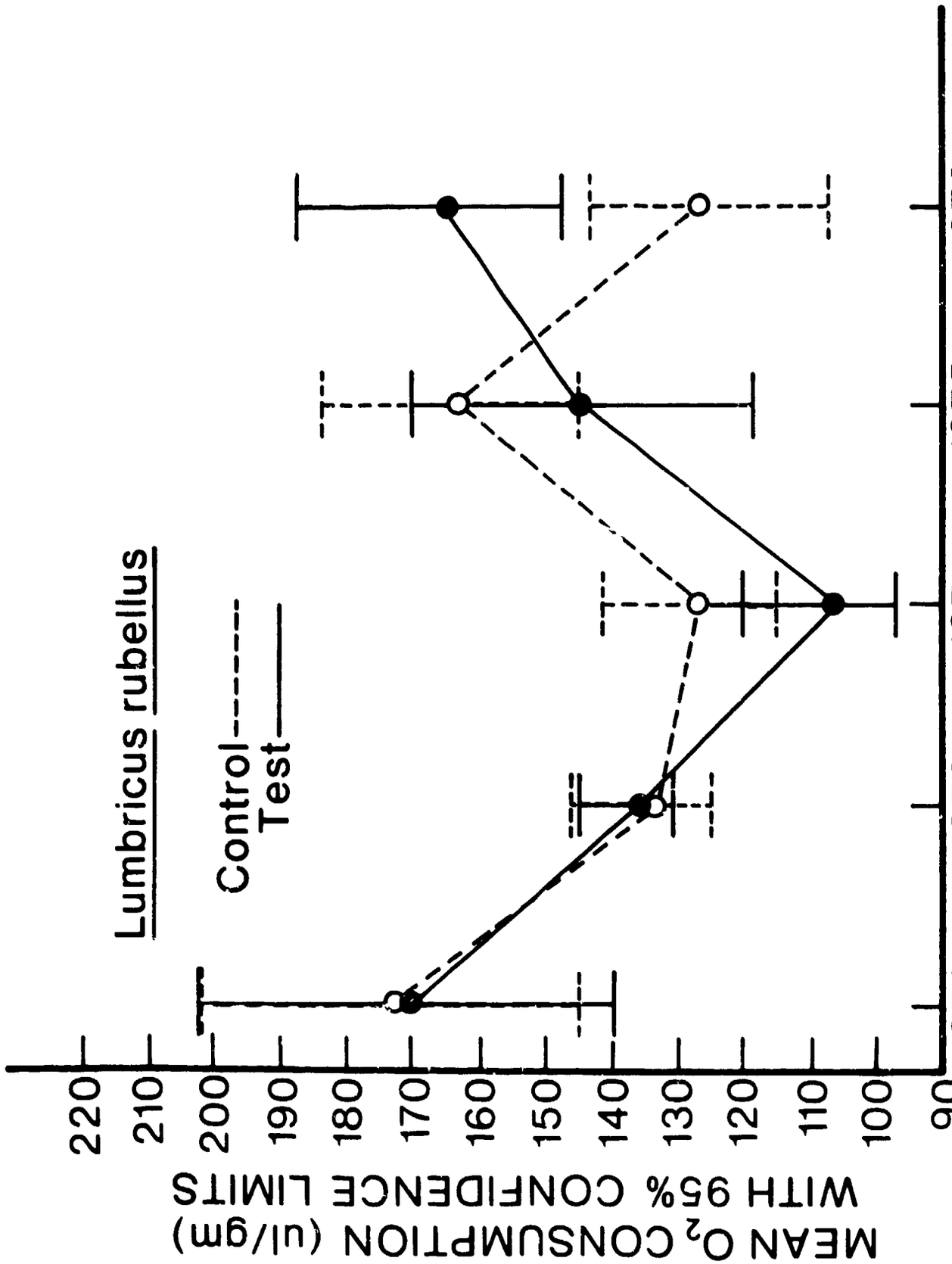

Fig. 3. Mean Oxygen Consumption of the Redworm, Lumbricus rubellus, 1972-76.

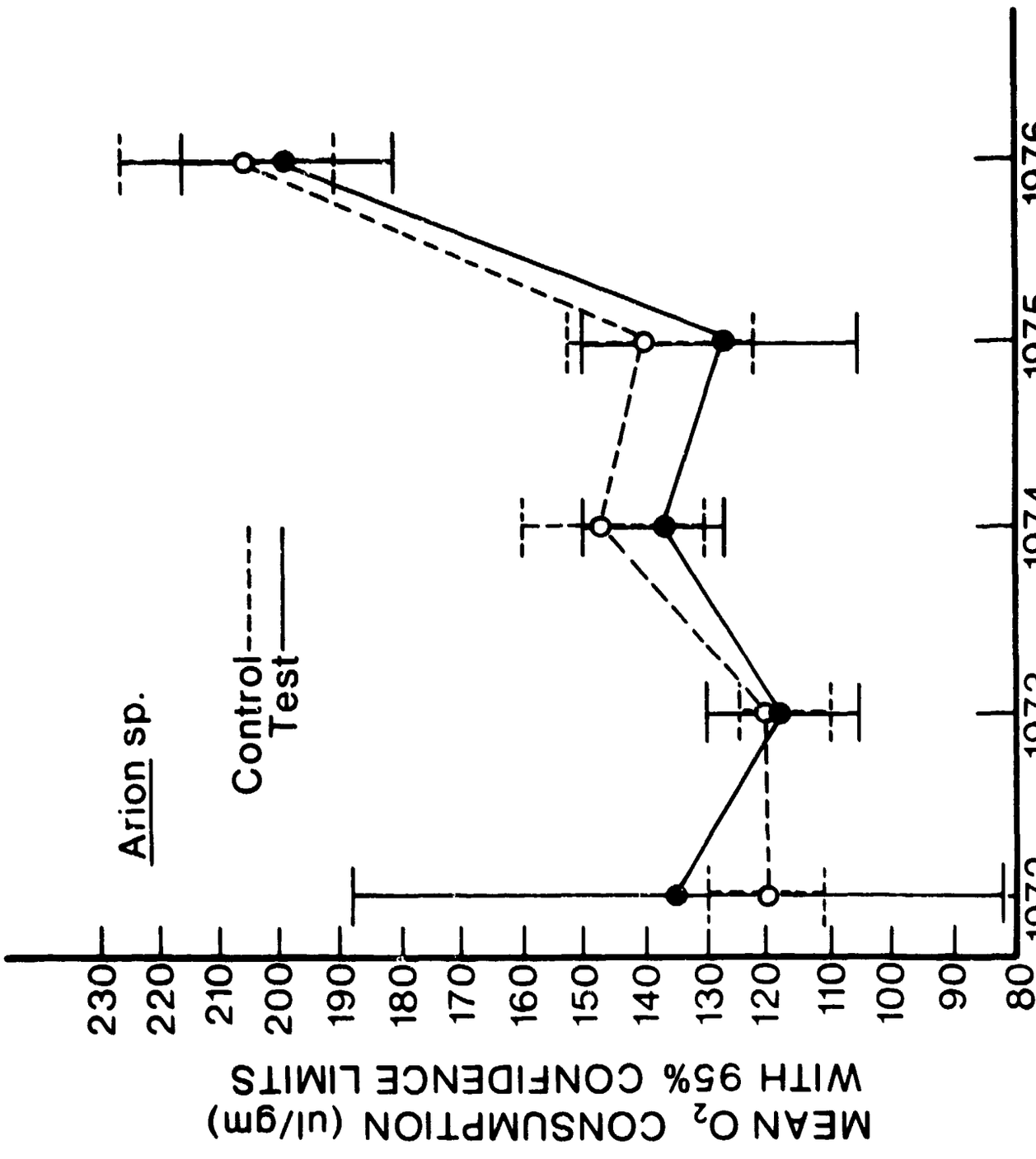

Fig. 4. Mean Oxygen Consumption of the Slug, Arion sp., 1972-76.

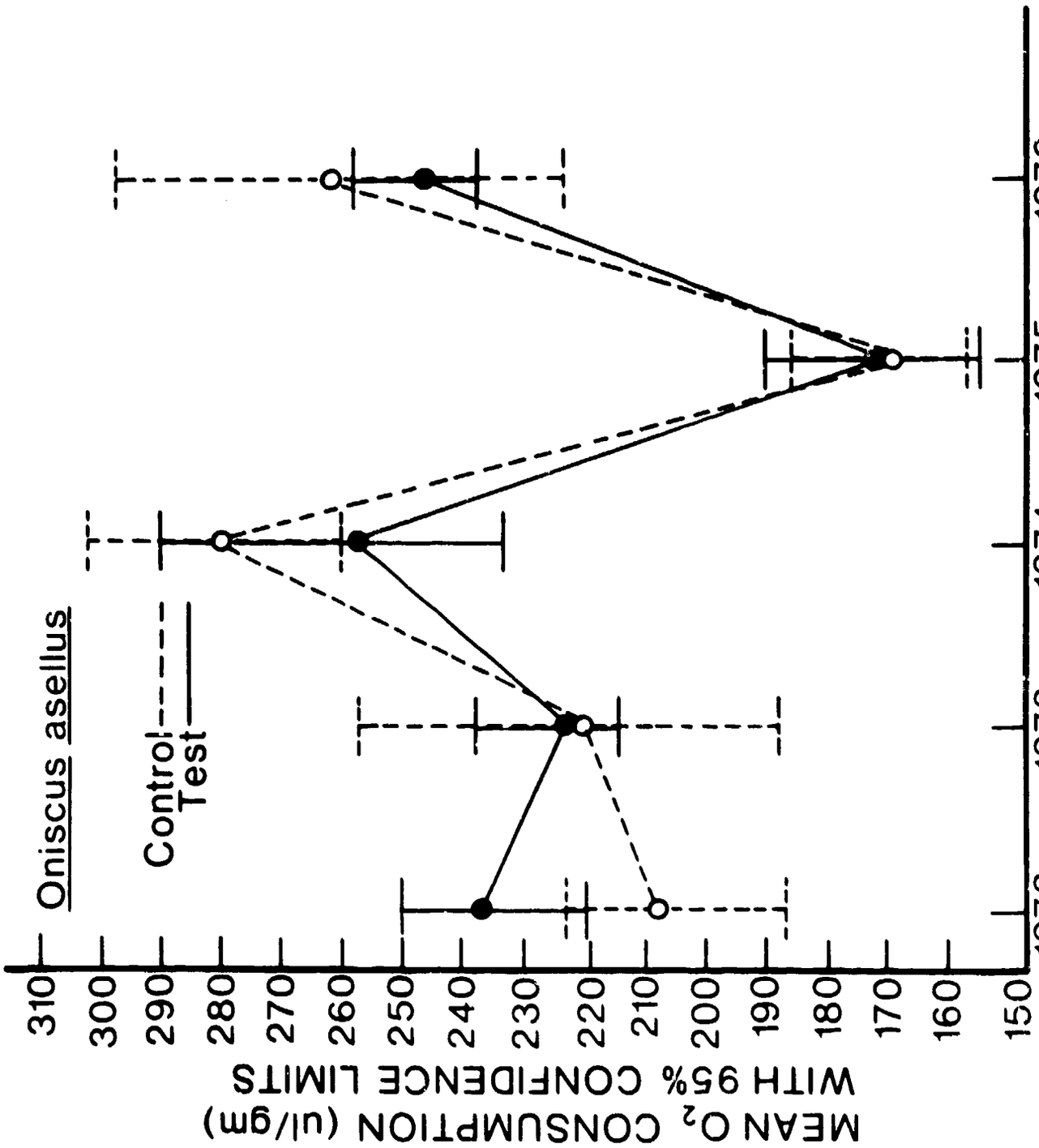

Fig. 5. Mean Oxygen Consumption of the Woodlouse, Oniscus asellus, 1972-76.

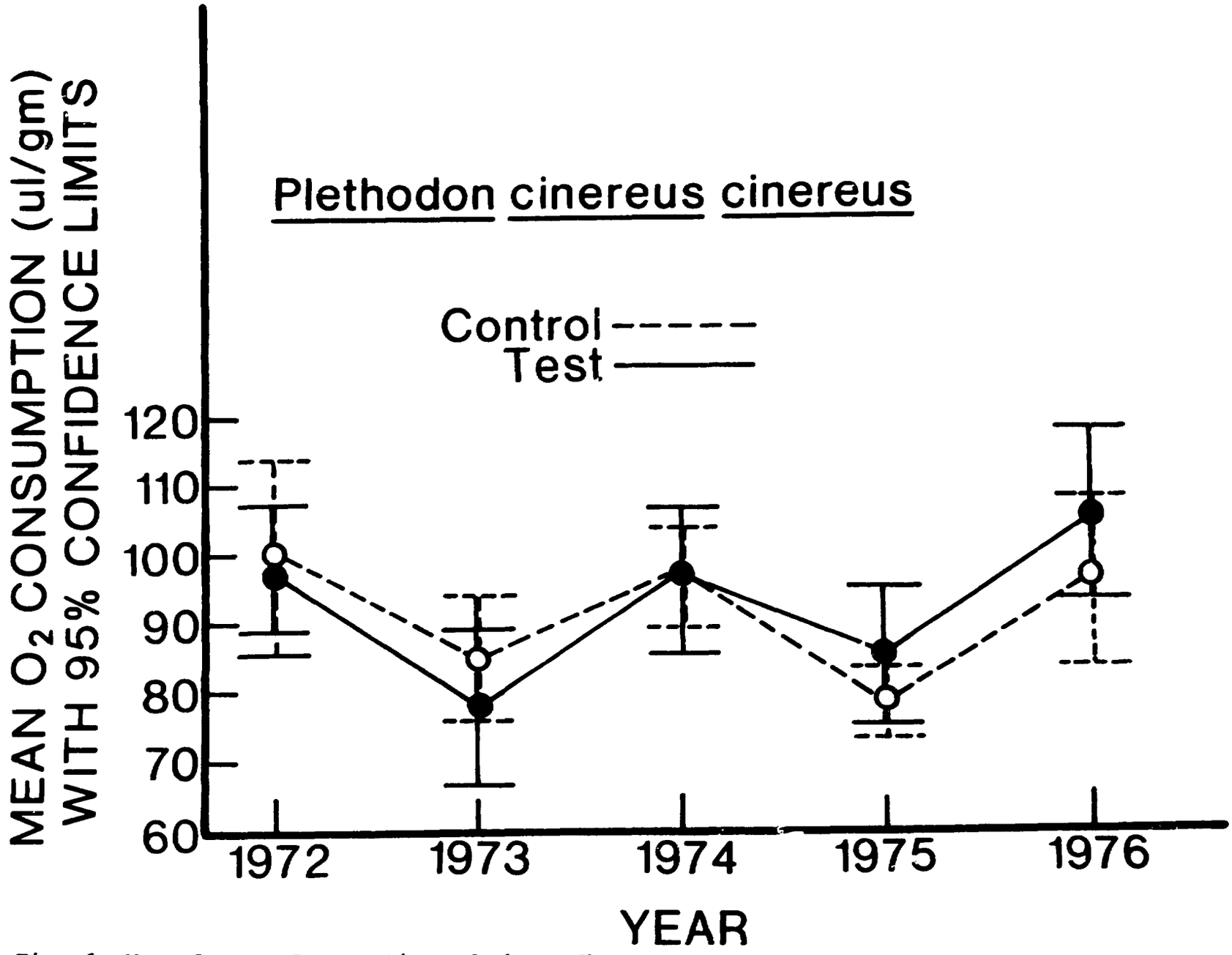

Fig. 6. Mean Oxygen Consumption of the Redbacked Salamander, Plethodon cinereus cinereus, 1972-76.

TABLE 6

PRECIPITATION (INCHES)

Year	May	June	July	August	Total
1972[+]	2.51	4.97	6.82	8.71	23.01
1973[+]	5.83	4.63	4.06	7.78	22.30
1975[+]	3.01	6.28	2.64	4.73	16.66
1976[0]	1.09	3.78	1.64	2.52	9.03

[+]Taken at Glidden

[0]Taken at WTF

TABLE 7

MEAN MAXIMUM AND MINIMUM TEMPERATURES (°F) FOR APPROXIMATELY 30 DAYS BEFORE COLLECTION OF SAMPLES[a]

Sample	1972		1973		1975		1976	
	Max	Min	Max	Min	Max	Min	Max	Min
Isopods	74	43	76	50	70	49	82	49
Earthworms	74	46	77	53	75	55	82	48
Redworms	77	53	77	55	77	52	84	50
Slugs	76	52	77	54	79	55	84	50
Salamanders	77	51	80	55	77	57	83	49

[a]Readings taken at WTF

preceding collection of the animals. It is readily seen that the summer of 1976 was hotter and drier than preceding years. Undoubtedly such strong environmental changes modify metabolic rates of poikilotherms but it is not possible to know what the effects might be without knowledge of each species' options and adaptations for dealing with temperature and dessication stress under natural conditions.

Another quantitative comparison between exposed and control animals is respiratory quotient (R.Q.), the ratio of oxygen consumed to carbon dioxide released (see Fig. 8). No significant differences were found in 1976, making a total of 46 out of 46 tests performed since 1972 which have not shown a significant difference in R. Q.

As in previous years, we did not observe any anomalies in the behavior, habitat selection, and general appearance and pigmentation of exposed animals in 1976.

TABLE 8

MEAN RESPIRATORY QUOTIENTS WITH 95% CONFIDENCE LIMITS OF EXPOSED AND CONTROL ANIMALS IN SUMMER 1976[a]

Organism	Exposed	Control
Earth-worms	0.82 (0.75 - 0.89)	0.82 (0.73 - 0.90)
Redworms	0.80 (0.74 - 0.85)	0.78 (0.74 - 0.82)
Slugs	0.77 (0.69 - 0.85)	0.73 (0.68 - 0.78)
Woodlice	0.81 (0.75 - 0.87)	0.78 (0.70 - 0.85)
Salamanders	0.82 (0.74 - 0.89)	0.84 (0.76 - 0.92)

[a]There were no significant differences between any exposed and control animals.

5. REFERENCES

Greenberg, B. 1974. Extremely Low Frequency Antenna Operation: Tests for Possible Impact on Five Naturally Exposed Animal Populations. Jour. Invert. Path., 23: 366-370.

Greenberg, B. 1976a. Metabolic Rates in Five Animal Populations after Long-term Exposure to Sanguine/Seafarer ELF Electromagnetic Fields in Nature. Final Report to IITRI, 29 pp.

Greenberg, B., and N. Ash. 1976b. Metabolic Rates in Five Animal Populations after Prolonged Exposure to Weak Extremely Low Frequency Electromagnetic Fields in Nature. Radiat. Res., 67: 252-265.

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Five species of woodland animals were collected during the summer of 1976 at the Wisconsin Test Facility (WTF). Field observations and sampling of the five exposed populations continue to show no abnormalities in behavior, habitat selection, or external features and pigmentation after seven years of WTF operation

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